

# Mutations in the *CYLD* gene in Brooke–Spiegler Syndrome, Familial Cylindromatosis, and Multiple Familial Trichoepithelioma: Lack of Genotype–Phenotype Correlation

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**Brooke–Spiegler syndrome (BSS), familial cylindromatosis (FC), and multiple familial trichoepithelioma (MFT), originally described as distinct entities, share overlapping clinical findings. Patients with BSS are predisposed to multiple skin appendage tumors such as cylindroma, trichoepithelioma, and spiradenoma. FC, however, is characterized by cylindromas and MFT by trichoepitheliomas as the only tumor type. These disorders have recently been associated with mutations in the *CYLD* gene. In this report, we describe three families with BSS, one with FC, and two with MFT phenotypes associated with novel and recurrent mutations in *CYLD*. We provide evidence that these disorders represent phenotypic variation of a single entity and lack genotype–phenotype correlation.**

**Key words:** *CYLD*/genodermatosis/mutation/tumor suppressor  
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Brooke–Spiegler syndrome (BSS, OMIM# 605041) (Brooke, 1892; Spiegler, 1899) is an autosomal dominantly inherited disease characterized by multiple skin appendage tumors. The predominating tumor can be a cylindroma, trichoepithelioma, and/or spiradenoma. Although cylindromas and spiradenomas are sweat gland tumors, trichoepitheliomas show hair follicle differentiation. Therefore, it has been postulated that BSS results from defects in the regulation of putative stem cells of the folliculo-sebaceous-apocrine unit, giving rise to different skin appendage tumors (Fenske *et al*, 2000). Two autosomal dominantly inherited genodermatoses, familial cylindromatosis (FC, OMIM# 132700), and multiple familial trichoepithelioma (MFT, OMIM# 601606), have been originally described as distinct entities. FC, originally known as Ansell–Spiegler cylindromas, is characterized by cylindromas, whereas MFT, originally referred to as Brooke–Fordyce trichoepitheliomas, is characterized by trichoepitheliomas (Welch *et al*, 1968).

The gene for FC was mapped to chromosome 16q12–q13 by linkage analysis (Biggs *et al*, 1995). The *CYLD* gene was discovered by positional cloning and germline mutations were identified in families with this disease (Bignell *et al*, 2000). Loss of heterozygosity at the *CYLD* locus has been found in both inherited and sporadic tumors, implicating *CYLD* as a tumor suppressor gene (Bignell *et al*, 2000). Subsequently, mutations in *CYLD* in patients with BSS phenotype were found (Hu *et al*, 2003). More recently, *CYLD* has been identified as the susceptibility gene for several families with MFT (Salhi *et al*, 2004; Zhang *et al*, 2004; Zheng *et al*, 2004). These findings, taken together

with the observations that features of BSS, FC, and MFT can occur in the same patient or in different patients within a single family (Gerretsen *et al*, 1995), and that a single *CYLD* mutation can be associated with both cylindromas and trichoepitheliomas (Poblete Gutierrez *et al*, 2002), suggest that these syndromes not only share a common genetic basis but represent phenotypic variation of the same disease.

To date, there have been 29 germline mutations described in the *CYLD* gene in families with BSS, FC, and MFT (Bignell *et al*, 2000; Poblete Gutierrez *et al*, 2002; Hu *et al*, 2003; Scheinfeld *et al*, 2003; Salhi *et al*, 2004; Zhang *et al*, 2004; Zheng *et al*, 2004). In this report, we describe three novel and two recurrent mutations in *CYLD* identified in five unrelated families with BSS, FC, and MFT phenotypes.

## Results

We evaluated three families with BSS, one with FC, and two with MFT phenotypes (Table S1). The pedigree of each family was consistent with an autosomal dominant mode of inheritance (data not shown). In all families except one, mutations in the *CYLD* gene were identified (Fig S1). These mutations are summarized in Table S1. All of the identified mutations were either nonsense or frameshift mutations resulting in premature stop codons. The case in which a mutation was not found (Cyld-3) showed an MFT phenotype with multiple trichoepitheliomas as the only skin tumor.

## Discussion

All of the reported mutations in *CYLD* have been heterozygous mutations with the majority (~90%) predicted

Abbreviations: BSS, Brooke–Spiegler syndrome; FC, familial cylindromatosis; MFT, multiple familial trichoepithelioma

to result in truncated proteins or lead to nonsense mediated mRNA decay. To date, no mutations in the N-terminal region of *CYLD* encoding the first two cytoskeletal-associated-protein-glycine-conserved motifs have been reported (exons 4–8). The absence of mutations in the N-terminal region encoding two CAP-GLY domains emphasizes the importance of these motifs for its function. The mutations, however, are scattered throughout the mid-portion and C-terminal region. Although clustering of mutations in the C-terminal region (exons 16–20) is noted ( $\sim 60\%$ ), this is not limited to one particular domain. As a result, for mutation screening strategies, we recommend starting with exons 16–20 and then pursuing the other parts of the gene.

Of interest, in one of the cases described in this report with the MFT phenotype (Cyld-3), we did not find sequence alterations in the coding sequence and flanking introns of *CYLD*. Although we cannot rule out the possibility of a large germline deletion involving *CYLD* in this case, it is possible that another locus for MFT exists. Evaluation of additional families with MFT will help clarify the presence of genetic heterogeneity.

In addition to tumors of the skin appendages, patients with BSS are also at risk for developing tumors of the major and minor salivary glands. Basal cell adenomas and adenocarcinomas of the parotid glands and minor salivary glands have been reported in association with this disease (Jungehulsing *et al*, 1999). One case described in this report (Cyld-6) demonstrated bilateral parotid gland basal cell adenocarcinomas, exemplifying this association.

We also evaluated the mutations in *CYLD* for genotype-phenotype correlation. To date, there have been six mutations described in association with the MFT phenotype. These mutations located in exons 10, 15–18, and 20 show no particular clustering in one region. Based on the current mutational data in individuals with the BSS, FC, and MFT phenotypes, in addition to the observation that within a single family some individuals may have trichoepitheliomas whereas others have cylindromas, it appears a correlation between genotype and phenotype is lacking.

### Materials and Methods

Six unrelated families with BSS, FC, or MFT phenotypes were recruited to this study. The Institutional Review Board at Columbia University approved all the described studies and the study was conducted according to Declaration of Helsinki Guidelines. Blood samples from affected individuals were collected after obtaining informed consent. Genomic DNA was extracted from whole blood using PureGene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota). The 17 coding exons of the *CYLD* gene were amplified by PCR using specific primers. For mutation detection, the PCR products were sequenced using an automated sequencing system (310, Applied Biosystems, Foster City, California).

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### Supplementary Material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23688/JID23688.htm>

**Figure S1.** Clinical findings and mutation analysis.

**Table S1.** Clinical data and mutation status of the families described in this report

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